

WHAT IS CLAIMED IS:

1. A method for constructing a normalized cDNA library of genes of low expression, comprising:

(a) constructing a non-normalized cDNA library from an RNA sample, wherein said RNA sample contains different species of RNA of different amounts, wherein said non-normalized cDNA library contains a plurality of members;

(b) separating the members of said non-normalized cDNA library;

(c) constructing a labeled probe library from said RNA sample;

10 (d) hybridizing a labeled probe library to said non-normalized cDNA library, whereby there is a differential of the amount of labeled probe of said labeled probe library hybridized to each individual member of said non-normalized cDNA library;

(e) identifying the individual members of said non-normalized cDNA library hybridized with low amounts of labeled probe; and

15 (f) pooling the individual members of said non-normalized cDNA library identified in step (e) in a collection;

whereby said collection is said normalized cDNA library of genes of low expression.

20 2. The method according to Claim 1, wherein said RNA sample is obtained from a cell.

25 3. The method according to Claim 2, wherein said RNA sample is a mRNA sample.

4. The method according to Claim 2, wherein said cell is an eubacteria, archaebacteria, or eukaryotic cell.

30 5. The method according to Claim 4, wherein said eukaryotic cell is a plant cell or animal cell.

6. The method according to Claim 5, wherein said plant cell is a soy, tobacco, wheat, rice, or corn cell.

7. The method according to Claim 5, wherein said animal cell is a human, 5 ape, mouse, rat, cow, pig, horse, goat, sheep, dog, cat, chicken, zebrafish, or fruitfly cell.

8. The method according to Claim 7, wherein said human cell is a human 10 kidney cell.

10 9. The method according to ~~Claim 1~~, wherein said normalized cDNA library is a normalized full-length cDNA library.

15 10. The method according to Claim 1, wherein said constructing comprises catalyzing a reverse transcription reaction for each species of said RNA sample, wherein said catalyzing takes place under conditions permissible for catalyzing a reverse transcription reaction.

20 11. The method according to Claim 10, wherein said catalyzing comprises:
(i) hybridizing poly-T oligonucleotide primers to said RNA sample;
(ii) adding dATP, dCTP, dGTP, dTTP, and reverse transcriptase; and
(iii) incubating said RNA sample at a temperature permissible for catalyzing a reverse transcription reaction.

25 12. The method according to Claim 1, wherein said non-normalized cDNA library is a non-normalized full-length cDNA library.

30 13. The method according to Claim 1, further comprising:
transforming each member of said non-normalized cDNA library into a host cell, wherein said transforming step is subsequent to said constructing and prior to said hybridizing.

14. The method according to Claim 13, further comprising: amplifying each member of said non-normalized cDNA library, wherein said amplifying comprises growing each said host cell containing, wherein said amplifying step is subsequent to said transforming and prior to said hybridizing.

15. A method for constructing a normalized cDNA library, comprising:

 - constructing a non-normalized cDNA library from an RNA sample, wherein said RNA sample contains different species of RNA of different amounts, wherein each member of said non-normalized cDNA library is separate from other members;
 - identifying the relative amounts of each member of said non-normalized cDNA library represented in said RNA sample;
 - dividing the members of said non-normalized cDNA library into groups; wherein one group of members of said non-normalized cDNA library is represented in low amounts by said RNA sample and one or more groups of members of said non-normalized cDNA library is represented in high amounts by said RNA sample;
 - selecting one group of said one or more groups of members of said non-normalized cDNA library represented in high amounts by said RNA sample;
 - identifying the members in said group of members that is not represented within a sub-group of members selected from said group of members;
 - forming a group of members from the members identified in step (e) and repeating step (e) until every member of said group of members has been selected within a sub-group of members;
 - repeating steps (d)-(f) with every group of said one or more groups of members of said non-normalized cDNA library represented in high amounts by said RNA sample;

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(h) pooling the members of said group of members of said non-normalized cDNA library represented in low amounts by said RNA sample and the members of every sub-group selected in a collection;
whereby said collection is said normalized cDNA library.

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16. The method according to Claim 15, wherein said RNA sample is obtained from a cell.

17. The method according to Claim 16, wherein said RNA sample is a 10 mRNA sample.

18. The method according to Claim 16, wherein said cell is an eubacteria, archaebacteria, or eukaryotic cell.

15 19. The method according to Claim 18, wherein said eukaryotic cell is a plant cell or animal cell.

20 20. The method according to Claim 19, wherein said plant cell is a soy, tobacco, wheat, rice, or corn cell.

21. The method according to Claim 19, wherein said animal cell is a human, ape, mouse, rat, cow, pig, horse, goat, sheep, dog, cat, chicken, zebrafish, or fruitfly cell.

25 22. The method according to Claim 21, wherein said human cell is a human kidney cell.

23. The method according to Claim 15, wherein said normalized cDNA library is a normalized full-length cDNA library.

30 24. The method according to Claim 15, wherein said constructing comprises catalyzing a reverse transcription reaction for each species of said RNA

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sample, wherein said catalyzing takes place under conditions permissible for catalyzing a reverse transcription reaction.

25. The method according to Claim 24, wherein said catalyzing comprises:
- 5 (i) hybridizing poly-T oligonucleotide primers to said RNA sample;
- (ii) adding dATP, dCTP, dGTP, dTTP, and reverse transcriptase; and
- (iii) incubating said RNA sample at a temperature permissible for catalyzing a reverse transcription reaction.
- 10 26. The method according to Claim 15, wherein said non-normalized cDNA library is a non-normalized full-length cDNA library.
- 15 27. The method according to Claim 15, further comprising: transforming each member of said non-normalized cDNA library into a host cell, wherein said transforming step is subsequent to said constructing and prior to said identifying of step (b).
- 20 28. The method according to Claim 27, further comprising: amplifying each member of said non-normalized cDNA library, wherein said amplifying comprises growing each said host cell containing, wherein said amplifying step is subsequent to said transforming and prior to said identifying of step (b).
- 25 29. The method according to Claim 15, wherein said identifying of step (b) comprises:
- (i) constructing a labeled probe library from said RNA sample;
- (ii) hybridizing said labeled probe library to said non-normalized cDNA library;
- (iii) identifying the relative amounts of labeled probe hybridized to each 30 member of said non-normalized cDNA library.

30. The method according to Claim 15, wherein said identifying of step (e) comprises:
- (i) constructing a labeled probe library from said sub-group of members;
- (ii) hybridizing said labeled probe library to said group of members;
- 5 (iii) identifying each member of said group of members that is not hybridized to by said labeled probe library.

31. The method according to Claim 15, further comprising:
- sequencing every member of said group members of said non-normalized
- 10 cDNA library represented in low amounts by said RNA sample and every member of every sub-group selected prior to said pooling, wherein a sufficient number of nucleotides are sequenced to identify members that are represented by more than once; and
- 15 pooling every unique member determined by said sequencing.

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32. A method for constructing a normalized cDNA library of genes of low expression, comprising:
- (a) constructing a non-normalized cDNA library from an RNA sample, wherein said RNA sample contains different species of RNA of different amounts, wherein each member of said non-normalized cDNA library is separate from other members;
- 20 (b) identifying the relative amounts of each member of said non-normalized cDNA library represented in said RNA sample;
- (c) pooling the members of said group of members of said non-normalized cDNA library represented in low amounts by said RNA sample in a collection;
- 25 whereby said collection is said normalized cDNA library of genes of low expression.

30. 33. A normalized cDNA library generated by the method of Claim 1.

34. A normalized cDNA library generated by the method of Claim 8.

35. A normalized cDNA library generated by the method of Claim 15.

36. A normalized cDNA library generated by the method of Claim 32.

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